

Presence, Introduction and Removal of Mutagenic Activity During the Preparation of Drinking Water in the Netherlands

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A survey of the presence of mutagenic activity in drinking water of 18 cities in the Netherlands revealed that in drinking water of 13 cities mutagenic activity could be demonstrated. The activity was detected in the Ames test after concentrating the organic mutagens with a XAD-4/8 procedure. Dose-related responses were observed with concentrates corresponding to 0.5 to 3.0 liters of drinking water.

A study of the changes in mutagenic activity during the preparation of drinking water in a few waterworks showed that breakpoint chlorination, transport chlorination and post chlorination increased the mutagenic activity, while ozonation only reduced the activity with metabolic activation. When adsorption on activated carbon powder was used, a certain reduction of mutagenic activity was observed. The use of activated carbon filters, however, removed the activity completely. The majority of organic mutagens present in drinking water concentrates were shown to be nonvolatile and relatively stable and probably consist of compounds with a molecular weight in the order of 200. These mutagens are not identical to the organics identified up till now in drinking water by standard gas chromatography/mass spectrometry analysis. Finally, a group of organic mutagens, which adsorbs only at pH 2-3 on XAD-4/8 (acid fraction), could be demonstrated in Ames-positive drinking waters.

Introduction

In the Netherlands, groundwater, surface water, and a mixture of both are used as raw water sources for drinking water. In particular, the rivers Rhine and Meuse serve as important sources for the drinking water supply; the two rivers provide the potable water for about 5 million people in the Netherlands (1).

In the Rhine and Meuse and in drinking water prepared from these rivers, several recognized mutagenic and carcinogenic organic compounds have been identified by Leer et al. (2), Morra et al. (3), and Zoeteman (4). Using the Ames Salmonella/

microsome assay in combination with the XAD concentration procedure for organics, mutagenic activity in 50 ml Rhine and 500 ml Meuse water was demonstrated by Van Kreijl et al. (5) and Kool et al. (6).

By using the same procedure in a limited drinking water survey in six cities, mutagenic activity was shown in 0.5 to 1 liter drinking water in four of these cities by Kool et al. (7). A more extended survey of the mutagenic activity in drinking water of 18 cities in the Netherlands was subsequently carried out in a collaborative program with the Dutch Waterworks and the Netherlands Waterworks' Testing and Research Institute (KIWA Ltd.). The selection of the cities has been based on the raw water source, the storage facility and the different treatment processes applied in the preparation of drinking water.

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In this paper the preliminary results of this survey are presented. In addition, results of a limited study are shown of the changes in mutagenic activity (introduction and removal) during the preparation of drinking water. Finally some characteristics of the organic mutagenic fraction present in drinking water are briefly discussed.

Materials and Methods

XAD Resins

Amberlite XAD-4 and 8 were obtained from Serva GmbH, Heidelberg F.R., Germany. Purification by repeated Soxhlet extraction, control by GC analysis and storage of the resins in methanol were described previously by Kool et al. (6).

XAD Concentration Procedure

Drinking water samples were taken just before the water left the treatment plant. The water was filtered (under nitrogen pressure) through a 0.45 μ m membrane filter before concentration. For about a 7×10^3 -fold concentration, 160 liters of the filtered water were passed over columns containing 20 cm³ XAD-4/8 at a flow rate of 4 bed volumes/min and at a constant temperature of 15°C. Elution of the adsorbed organic constituents took place with an appropriate volume of DMSO or acetone (≥ 1 bed volume). For lower or higher concentration factors, corresponding smaller or larger volumes of water were passed through the XAD column until the desired water/eluante ratio (v/v) was obtained.

Thin Layer Chromatography (TLC)

For the TLC fractionation of acetone concentrates of drinking water, preparative plates precoated with 2.0 mm of Silicagel-G (PSG Merck, Fertigplatten) were used. A volume of 2.0 ml acetone concentrate was applied to the plate with an automatic spraying device in the form of a small band (4.0 \times 0.3 cm). The chromatograms were developed in one direction using ethyl acetate:isooctane (1:1) as the first solvent system. The plates were air-dried overnight and a second solvent system, benzene:methanol (4:1), was used. The plates were air-dried again prior to further investigation.

The developed plates were examined under ultraviolet light (366 nm) in order to mark the separate bands. The marked fractions (six) were collected by scraping off and collecting the adsorbents with a Pasteur pipet connected to a vacuum pump. The outlet of the pipet was fitted with a plug of glass wool. The organic material was recovered by eluting the pipet with 5 ml DMSO. The eluates were stored at -20°C prior to mutagenicity testing.

Gel Filtration on Sephadex LH20

The glass column (height 40 cm, 1 cm) was packed with Sephadex LH20 in dioxane-water (7:3) as described by Concini et al. (8). About 1.0 ml of a DMSO/XAD concentrate of drinking water was layered on the column, and subsequent gel filtration was performed with dioxane-water (7:3) as solvent. Fractions of 1 ml were collected with an automatic fraction collector. After measuring the absorbance at 263 nm, the fractions were pooled, diluted five-fold in water, reconcentrated on XAD-4/8 (bed volume 4 ml), and eluted with 5 ml DMSO. The concentrate was stored at -20°C prior to mutagenicity testing. Calibration of the column was performed by using two colored markers: Vitamin B₁₂ (molecular weight 1355) and nitrofurazon (molecular weight 198).

Bacterial Strains

The *Salmonella typhimurium* strains TA 98 and TA 100 as described by Ames et al. (9) were used. They were stored frozen at -80°C in nutrient broth containing 10% DMSO.

Ames Salmonella/Microsome Assay

Mutagenicity testing of the organic concentrates of drinking water was carried out according to the plate incorporation assay described by Ames et al. (9).

The induction of microsomal enzymes with Aroclor 1254 and the preparation of the rat liver homogenates (S-9) has also been described by Ames et al. (9). In the S-9 mix, 0.075 ml of liver homogenate was added per milliliter of mix. All water concentrates were tested in three to five replicates, and the results were considered significant when a two-fold increase above the background as well as dose response effects were observed. The deviation of the mean was usually below 20%. Routine controls to check for the presence of factors affecting bacterial growth were incorporated as described by Kool et al. (6).

Table 1. Types of drinking water investigated.

City number	Drinking water prepared from
1-5	Ground water
6	Dune-infiltrated Meuse water
7, 8a, 9	Dune-infiltrated Rhine water
10-15	Bank-infiltrated Rhine water
16	River Meuse water
8b, 17, 18	River Rhine water

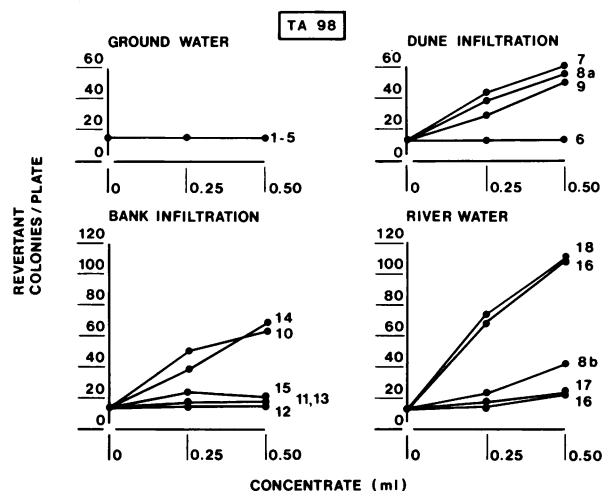


FIGURE 1. Mutagenic activity in drinking water of 18 cities in the Netherlands. The sampling, 7000-fold concentration with XAD-4/8, elution with DMSO and subsequent mutagenicity testing with *S. typhimurium* strains TA 98 and TA 100 are described in the text. The numbers refer to the 18 cities listed in Table 1, and each point represents the average of five plates.

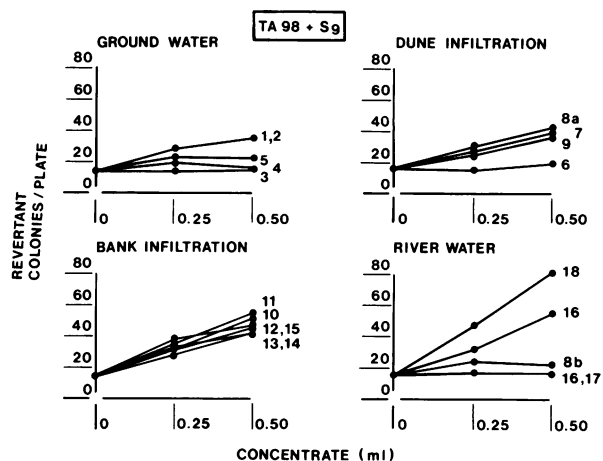


FIGURE 2. Mutagenic activity in drinking water of 18 cities in the Netherlands. Sampling, 7000-fold concentration with XAD-4/8, DMSO elution and mutagenicity testing as described in the text. The numbers refer to the 18 cities listed in Table 1, and each point represents the average of five plates.

Results

Mutagenic Activity of Drinking Water in 18 Cities

Our previous results, showing mutagenic activity in drinking water, all relate to drinking water prepared from the rivers Rhine and Meuse (7). To investigate whether these results are representative

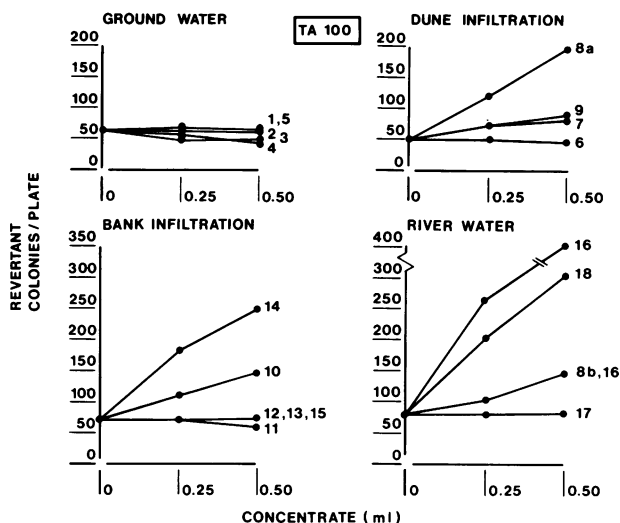


FIGURE 3. Mutagenic activity in drinking water of 18 cities in the Netherlands. Sampling, 7000-fold concentration with XAD-4/8, DMSO elution and mutagenicity testing as described in the text. The numbers refer to the 18 cities listed in Table 1, and each point represents the average of five plates.

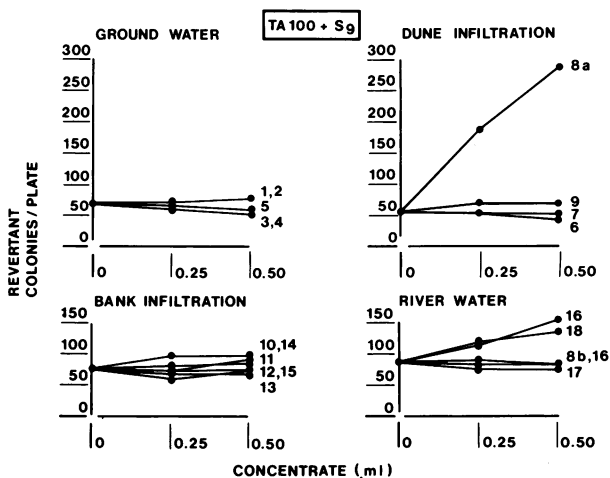


FIGURE 4. Mutagenic activity in drinking water of 18 cities in the Netherlands. Sampling, 7000-fold concentration with XAD-4/8, DMSO elution and mutagenicity testing as described in the text. The numbers refer to the 18 cities listed in Table 1, and each point represents the average of five plates.

for other types of drinking water in the Netherlands, an extended survey in 18 cities (20 types of drinking water) was carried out. As shown in Table 1, these cities prepare their drinking water either from groundwater, surface water, or a mixture of both. In city 8 and city 16, drinking waters after two different treatment processes were investigated.

The results of the survey are presented in Figures 1–4 and summarized in Table 2. When the

Table 2. Summary of mutagenic activity present in 20 types of drinking water.

Raw water source	Type of storage facility	Number of drinking water investigated	Number of cities positive in the Ames test ^a			
			TA 98	TA 98 + S9	TA 100	TA 100 + S9
Ground water	—	5	0	2(1,2)	0	0
River/ground water	Dune infiltration	4	3(7, 8a, 9)	3(7, 8a, 9)	1(8a)	1(8a)
River/ground water	Bank infiltration	6	2(10, 14)	6(10-15)	1(14)	0
River water	Storage reservoir	5	3(8b, 16, 18)	2(16, 18)	2(16, 18)	2(16, 18)

^aNumbers in parentheses are city numbers.

cities are classified in four groups with regard to the raw water source and type of treatment, several interesting features are observed. Firstly, only two out of the five cities which prepare their drinking water solely from groundwater show low but significant mutagenic activity. Secondly, drinking waters (12 out of 15) in cities which prepare their drinking water from surface water or (in)filtrated surface water show mutagenic activity at least in one of the strains used.

Only one city in the categories "dune infiltration" (city 6) and "surface water" (one drinking water type of city 16) shows no mutagenic activity. Thirdly, the level and type of mutagenic activity observed seems to be more dependent on the type of treatment than of the type of raw water.

The results obtained showed to be very reproducible, since a second survey carried out six months later gave almost identical results (not shown).

Introduction and Removal of Mutagenic Activity During the Preparation of Drinking Water

The rivers Rhine and Meuse contain mutagenic activity as shown previously by Van Kreijl et al. (5) and Kool et al. (6). They demonstrated that this activity predominantly requires metabolic activation (S-9 mixture). In drinking water, however, the mutagenic activity is most pronounced without metabolic activation in many of the cities. This suggests that during drinking water preparation a change in mutagenic activity has occurred. Therefore, we studied the changes in the mutagenic activity in some of the waterworks. The effects of chlorine treatment (breakpoint chlorination, transport chlorination, post chlorination), ozonization, activated carbon adsorption and dune infiltration were examined. The results are shown in Figures 5-7.

Figure 5 (city 16) shows that the type of mutagenic activity of the raw water source is still present after storage for about 100 days in a reservoir. After breakpoint chlorination, however, the direct acting mutagenic activity in both strains was greatly

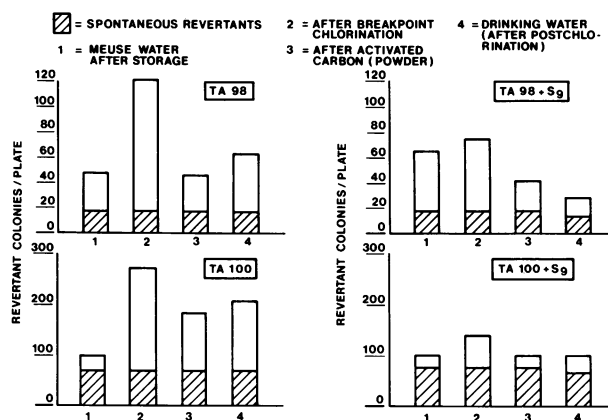


FIGURE 5. Course of mutagenic activity during the preparation of drinking water in city 16. The sampling, 7000-fold concentration of water samples, DMSO elution and Ames testing are as described in the text. The results correspond to about 1.5 liter of water per plate.

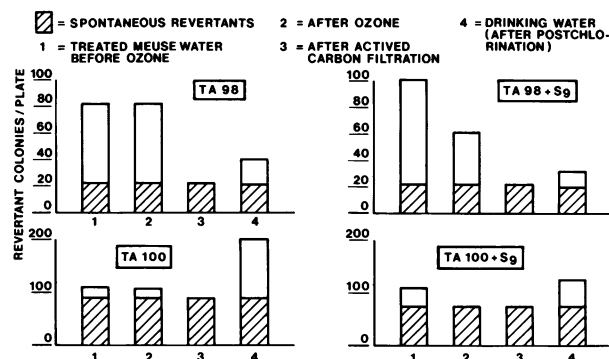


FIGURE 6. Course of mutagenic activity during the preparation of drinking water in city 16. The sampling, 7000-fold concentration of water samples, DMSO elution and Ames testing are as described in the text. The results correspond to about 3 liters of water per plate.

increased. Activated carbon (powder) reduces the mutagenic activity in all cases but the reduction is less marked in strain TA 100. The slight increase of the direct acting mutagenic activity in both strains in finished drinking water may be due to post

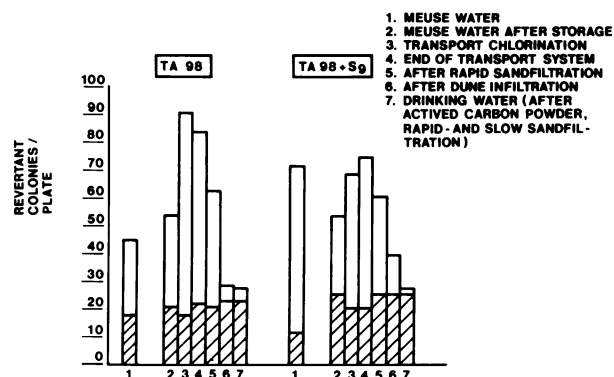


FIGURE 7. Course of mutagenic activity during the preparation of drinking water in city 6. The sampling, 8000-fold concentration of water samples, DMSO elution and Ames testing are as described in the text. The results correspond to about 2 liters of water per plate.

chlorination. Figure 6 (city 16) shows that an ozone treatment does not reduce the direct acting mutagenic activity with strain TA 98 and only partly reduces the activity with TA 98 + S-9. In this case the original mutagenic activity with TA 100 \pm S-9 is so marginal, that no conclusion can be drawn with regard to the ozone treatment.

The mutagenic activity is completely removed in both strains by activated carbon filtration, while post chlorination introduces mutagenic activity in both strains again. Figure 7 (city 6) shows that transport chlorination also increases the mutagenic activity with strain TA 98 (\pm S-9). No activity was observed with strain TA 100. Rapid sand filtration hardly reduced the activity while dune infiltration reduced the mutagenic activity to a great extent. Finally, the residual mutagenic activity is completely removed by adsorption on activated carbon (powder) in combination with a rapid and slow sand filtration step.

Characterization of the Mutagenic Fraction and Some Properties of the Responsible Organic Mutagens

By using the XAD-4/8 procedure for concentrating organic mutagens in drinking water, mutagenic activity can be detected in 13 out of 18 cities. Readsorption of the remaining compounds in the drinking water of city 18 (after passing the XAD column) on a second column at pH 2-3, however, revealed the presence of another class of organic mutagens (acid fraction) (10). Whether this class of organic mutagens generally is present in drinking water was investigated. Four drinking waters positive in the Ames test and one negative were stud-

ied. The four drinking waters with mutagenic activity also contained this class of organic mutagens which only adsorbs at pH 2-3, while the drinking water negative in the Ames test did not (not shown).

At present there is little information on the nature of the organic mutagens present in drinking water. Therefore, investigations are carried out to determine the chemical composition of the mutagenic fraction obtained by the XAD concentration procedure. We have reported previously (10) that the major part of the organic mutagens in city 18 are in the slightly polar nonvolatile fraction. They are still present after boiling the water and are not identical with the organic compounds already identified in drinking water by routine GC/MS analysis.

In an attempt to fractionate the organic mutagens, thin layer chromatography of acetone concentrates derived from stored surface water and drinking water prepared from this surface water has been carried out. The results in Figure 8 show that the organic mutagens active on strain TA 98 which are present in surface and drinking water predominantly are found in fraction 3. The results after TLC analysis suggest that the direct acting organic mutagens present in fraction 3 of the surface water concentrate are largely recovered in fraction 3 of the drinking water concentrate. The organic mutagens requiring metabolic activation in surface water, however, are more efficiently removed by the purification processes. The presence of organic mutagens which show an identical migration and fluorescence pattern on the thin layer plate suggests that the mutagenic compounds present in both fractions 3 may be the same. To estimate the molecular weight of the organic mutagens in this fraction, gelfiltration of Sephadex LH20 was carried out.

The results shown in Figure 9 indicate that the majority of the organic mutagens present in TLC fraction No. 3 has a molecular weight in the order of 200 assuming that these organics show the same behavior in Sephadex LH20, as the controls.

Discussion

In the Netherlands a limited survey by Kool et al. (7) on mutagenic activity in drinking water revealed that in four out of six cities mutagenic activity could be shown. All cities in this survey, however, prepare their drinking water from surface water.

To see whether these results are representative for drinking water in the Netherlands, a survey in 18 cities has been carried out including groundwater, surface water, and mixtures of both as raw water source. It was found that in 13 cities mutagenic activity could be demonstrated in 0.5 to 3 liters of drinking water (Figs. 1-4). The results of

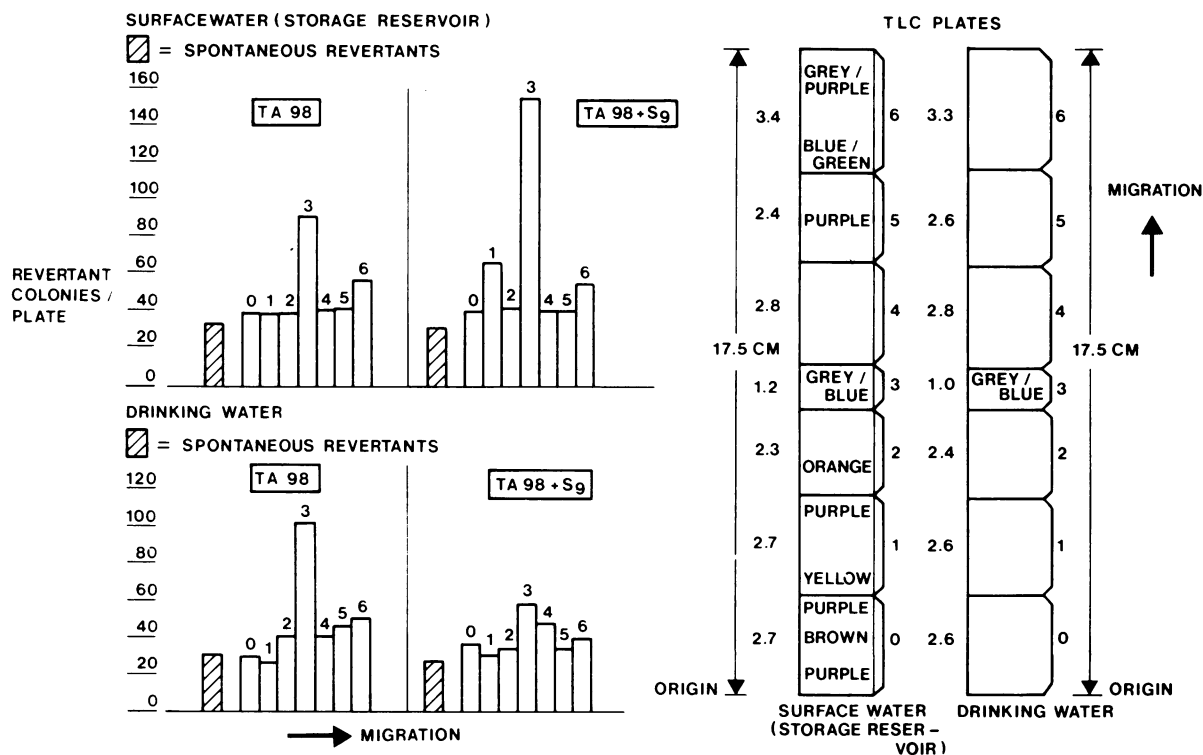


FIGURE 8. Fractionation of water concentrates prepared from storage reservoir water and drinking water by thin layer chromatography. The sampling, 9000-fold concentration of water samples from city 18, and elution with 60 ml acetone are as described in the text. The acetone concentrates were mixed with four volumes of H_2O and re-concentrated on a smaller XAD-4/8 column and again eluted with acetone (12 ml). These acetone concentrates (45,000-fold concentration) were tested by TLC. After incubation, six fractions were scraped off the TLC plate and eluted with 6 ml DMSO and assayed in the Ames test ($TA\ 98 \pm S-9$).

this survey are not incidental because similar results in the 18 cities were obtained during a second survey six months later.

From these results it appeared that two out of the five cities which use groundwater as raw water source showed mutagenic activity, but this activity was only marginal (Fig. 2). Most cities (12 out of 15) which prepare their drinking water from surface water or infiltrated surface water clearly showed mutagenic activities. It is interesting that all cities which use bank-infiltrated river water as raw water source were positive in the Ames test with strain TA 98 + S-9 and that only one city in the dune infiltration category (city 6) and one (drinking water type of city 16) in the river water category did not show mutagenic activity in the Ames test. For further information about bank and dune infiltration see Piet and Zoeteman (11).

From these results the conclusion can be drawn that a proper combination of treatment processes may remove the organic mutagens to a high degree. The finding that mutagenic activity in Dutch surface water predominantly requires metabolic activation,

while the activity in drinking water in many cities is most pronounced without metabolic activation (Figs. 1-4) indicates that a shift in the overall type of activity may have occurred due to different treatment processes. Cheh et al. (12) and de Greef et al. (13), who investigated whether the water quality will change after a chlorine treatment, reported an increase of mutagenic activity. Bull et al. (14) discussed the evidence for an increase of carcinogenic activity in chlorine treated water. To see how the changes in mutagenic activity take place in practice, several purification processes applied in different waterworks were investigated. In particular, a breakpoint chlorination dramatically increases the mutagenic activity (Fig. 5). However, transport chlorination (Fig. 7) as well as post chlorination (Fig. 6) do increase the mutagenic activity to a lesser extent. Ozonization of the water decreased the indirect mutagenic activity on strain TA 98, while the activity remained unchanged without metabolic activation (Fig. 6).

Considering the treatment with activated carbon powder, it is obvious that the removal of mutagenic

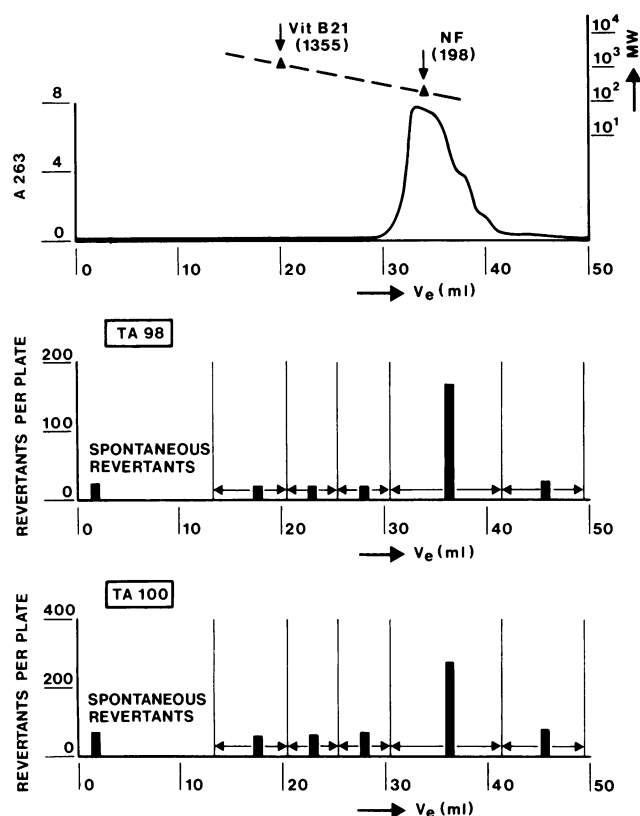


FIGURE 9. Gel filtration of a drinking water concentrate (city 18) with Sephadex LH20. Sampling, 250,000-fold concentration of drinking water on XAD-4/8, elution with DMSO and subsequent gel filtration were as described in the text. After measuring the absorbance at 263 nm, the fractions were pooled and, after dilution in water, reconstituted on XAD-4/8, eluted with 5 ml DMSO and assayed for mutagenic activity with *S. typhimurium* strains TA 98 and TA 100.

activity is not very efficient (Fig. 5). Activated carbon filters, however, were shown to be very efficient in the removal of organic mutagens, because no mutagenic activity could be detected in the water with the Ames test after this step. Other activated carbon filtration experiments with six different activated carbon filters showed equivalent results (Kool, unpublished data).

Finally, dune infiltration in combination with activated carbon (powder) and slow sand filtration were shown to be very effective in a waterwork in removing organic mutagens present in water (Fig. 7).

Up to now there has been little information on the nature of the mutagenic compounds in drinking water. Recently, however, Tabor and Loper (15) reported that mutagenic activity in a drinking water concentrate from Cincinnati may be due to the presence of isomeric chlorinated aliphatic ethers. Kool et al. (7) found that the mutagenic activity of a

Dutch drinking water is observed mainly in the slightly polar nonvolatile fraction and that these compounds are not the same organics already identified in this type of drinking water by GC/MS.

These results indicate that these organic mutagens are not the same compounds as described by Tabor and Loper (15). Kool et al. (10) also observed another class of organic mutagen present in one type of drinking water. The presence of this class of mutagens (only adsorbed at pH 2–3 on XAD 4/8) was also detected in other Ames positive drinking waters. The behavior of this class of organic mutagens (acid fraction) during different water purification processes is under investigation.

Using thin layer chromatography we showed that the direct acting mutagenic fraction on TA 98 present in surface water is found at the same level in drinking water (Fig. 8). Furthermore, preliminary experiments using gel filtration on Sephadex LH20 with a DMSO concentrate of drinking water indicate that these organic mutagens probably have a molecular weight of the order of 200 (Fig. 9).

Finally the presence of mutagenic activity in the Ames test in drinking water of 13 cities raised the question whether these findings are significant for human health. To answer this question more research has to be carried out. Long-term animal studies with drinking water concentrates as well as additional *in vitro* biological assays and chemical analysis are presently carried out on the mutagenic fractions in order to obtain a better understanding of their health significance.

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